Nucleic acid techniques

Plasmid DNA and DNA restriction fragments were isolated and analyzed by standard methods (Sambrook, J. et al. (1989) Molecular cloning; a laboratory manual. 2nd edition. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.). Restriction enzymes, ligases, and other enzymes were used according to the manufacturer's instructions.

Physical characterization of poly(4-hydroxybutyric acid) samples

Analysis of polymer composition was performed using gas chromatography methods as described by Braunegg, G. et al. (1978, *Eur. J. Appl. Microbiol.* 6:29-37). Analysis of molecular weight and polydispersity was performed by gel permeation chromatography (GPC, Ishihara, Y. et al. (1996) *J. Ferm. Bioeng.* 81:422-428). Analysis of melting points, rate of crystallization, dH_m, and E_a were performed by differential scanning calorimetry (DSC, Kemnitzer, J.E. et al. (1995) *J. Env. Polym. Degrad.* 3:37-47).

EXAMPLE 1: Construction of plasmids

A 3.5-kbp *SmaI/ApaI* restriction fragment comprising the entire polyhydroxyalkanoic acid synthase structural gene (*phaC*$_{Ae}$, GenBank Accession number J05003, Peoples, O.P. and Sinskey, A.J. (1989) *J. Biol. Chem.* 264:15298-15303) plus 878 of 1,221 bp of the 5' region of the β-ketothiolase structural gene from *A. eutrophus*, referred to as SA35, was isolated from the hybrid plasmid pSK2665 that had been cloned previously (Schubert, P. et al. (1991) *J. Bacteriol.* 173:168-175). In addition, a 1.8 kb *ApaI*/*Eco*RI restriction fragment comprising the entire orfZ$_{Ck}$ (*phaA'*$_{Ae}$, GenBank Accession number L21902, Söhling, B. and Gottschalk, G. (1993) *J. Bacteriol.* 178:871-880) from *C. kluyveri*, and referred to as AE18, was isolated from the hybrid plasmid pCK3pSK that had been cloned previously (Söhling, B. and Gottschalk, G. (1996) *J. Bacteriol.* 178:871-880). Both fragments were ligated to EcoRI/SmaI digested pBluescript vectors KS$^-$ and SK$^-$. The ligation products (pKSSE5.3 and pSKSE5.3, respectively) were transformed into *Escherichia coli* strain XL1-Blue using calcium chloride methodologies (Sambrook, J. et al. (1989) Molecular cloning; a laboratory

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